Gibson Assembly

Overview :

Gibson Chew Back and Anneal Assembly (Gibson CBA) is a quick and easy method to construct plasmids without using restriction enzymes. In this method, DNA fragments to be assembled are PCR amplified with 40 bp of overlap to the adjacent sequence. These fragments are then mixed in a single pot with a single strand exonuclease to generate sticky ends and allowed to anneal before being repaired by a polymerase and a ligase.

Procedure :

5x Isothermal Reaction Mix

Composition	Volume	Final Concentration
Tris-HCl pH:7.5	3 mL	500 mM
$MgCl_2$	150 µL	50 mM
dNTP	60 µL	1 mM
PEG-8000	1.5 g	25 %
NAD	300 µL	5 mM
H_20	6 mL (final)	-

Assembly Master Mix

Composition	Volume
5X Enzyme Mix	320 µL
T5 exconuclease	0.64 µL
Phusion polymerase	20 µL
Taq ligase	160 µL
H20	1.2 mL (final)

1. Vector and insert(s) ensuring that at least 40 bp homology exists between adjacent fragments

2. Thaw assembly Master Mix and keep on ice until ready to be used

3. Mix 15 µl of Assembly Mixture with 5 µl total of cleaned PCR product keeping DNA inserts in equimolar amounts

4. Incubate at 50 °C for 15-60 min (60 min optimal).

5. Transform cells with no more than 1 µl of Assembly Mixture.